#929

PHARMACOLOGY

Comm.
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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

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NEW YORK, N. Y. 10022
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JUL 30 1973

Application for Research Grant
(Use extra pages as needed)

Date 7.25.73

1. Principal Investigator (give title and degrees):
Edward Leete, Professor of Chemistry, B.Sc., Ph.D., D.Sc.

2. Institution & address:
University of Minnesota,
Minneapolis
Minnesota, 55455.

3. Department(s) where research will be done or collaboration provided:
Department of Chemistry

4. Short title of study:
Synthesis and Biological Activity of Nicotine Analogs

5. Proposed starting date: 1.1.74

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

Analogs of nicotine and related tobacco alkaloids will be synthesized for pharmacological testings . Substituted nicotine derivatives will be prepared with known stereochemistry in an effort to determine the relationship between biological activity and the conformation of the nicotine molecule. Biological testing will be carried out by Professor U. S. von Euler and his associates at the Karolinska Institute, Stockholm.

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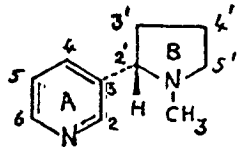
8. Brief statement of working hypothesis:

From work already published (cf. F. Haglin, Acta Pharm. Suecica, 4, 117 (1967)) it is clear that certain structural features are required in a molecule if it is to exhibit "Nicotinic Activity" in biological tests. The pyridine ring (A) is essential, however it is possible to substitute this ring at the 5- and 6-positions and retain activity. Substitution at the 2- and 4-positions results in a drastic decrease in activity, and it is suggested that such substitution results in restricted rotation around the C-3~C-2' bond, inhibiting the required interaction of the nicotine molecule with the receptor site (presumably on some membrane) which leads to the biological properties of nicotine. Initially it is proposed to substitute the pyrrolidine ring (B) with methyl groups, having known stereochemistry relative to the pyridine ring The biological activity of these analogs will be compared with l-nicotine.

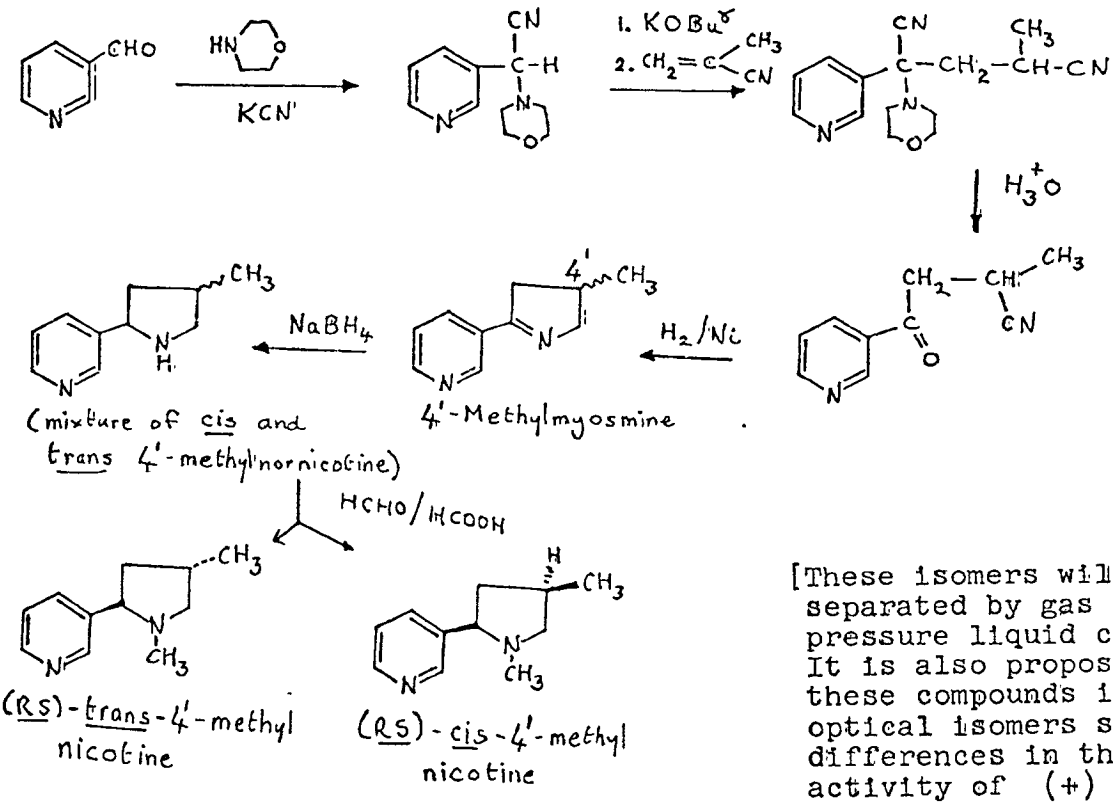


l-Nicotine

9. Details of experimental design and procedures (append extra pages as necessary)

We have recently developed a new synthesis of nornicotine and myosmine (E. Leete, M. R. Chedekel, and G. B. Bodem, J. Org. Chem., 37, 4465 (1972)) which we feel will be of general use for the synthesis of large amounts of nicotine analogs. The synthetic schemes which we propose to use for the preparation of these analogs are illustrated below.

4'-Methylnicotine



[These isomers will be separated by gas or high pressure liquid chromatography It is also proposed to separate these compounds into their optical isomers since differences in the biological activity of (+) and (-) nicotine has been observed (R. B. Barlow and J. T. Hamilton, Brit. J. Pharm. Chem., 25, 206 (1965).]

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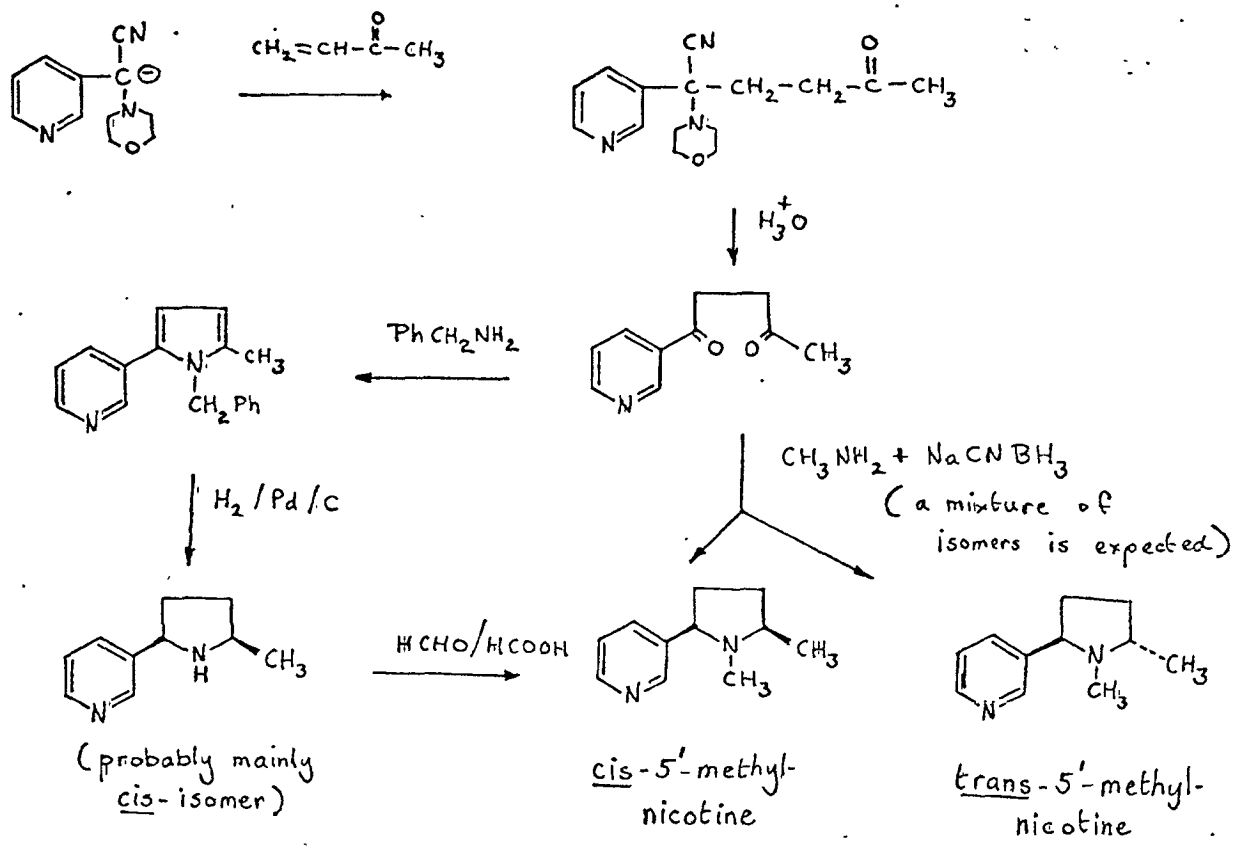
Item 9 (continued)

5'-Methylnicotine

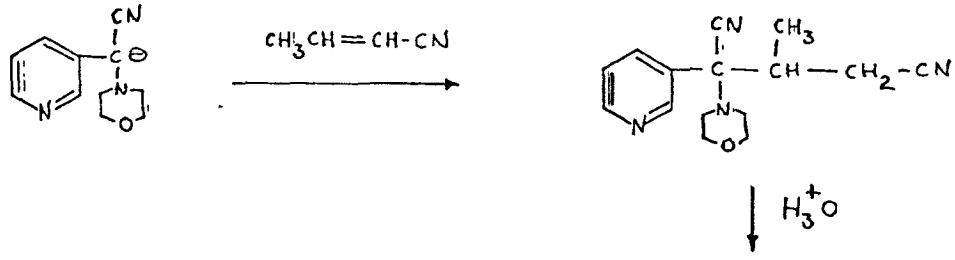
This compound has been described by I. Yamamoto (Agr. Biol. Chem. (Tokyo), 27, 445 (1963)), however the stereochemistry of the methyl group was not determined and presumably a mixture of isomers was obtained.



The 5'-dimethylnicotine has been prepared by Castagnoli (J. Pharm. Sci., 58, 860 (1969)) and A. Burger (J. Pharm. Sci., 59, 342 (1970) .

3'-Methylnicotine

The trans-isomer of this compound has been prepared by Castagnoli (J. Org. Chem., 37, 1268 (1972)) . It is expected that the following method will furnish both the cis and the trans isomers.



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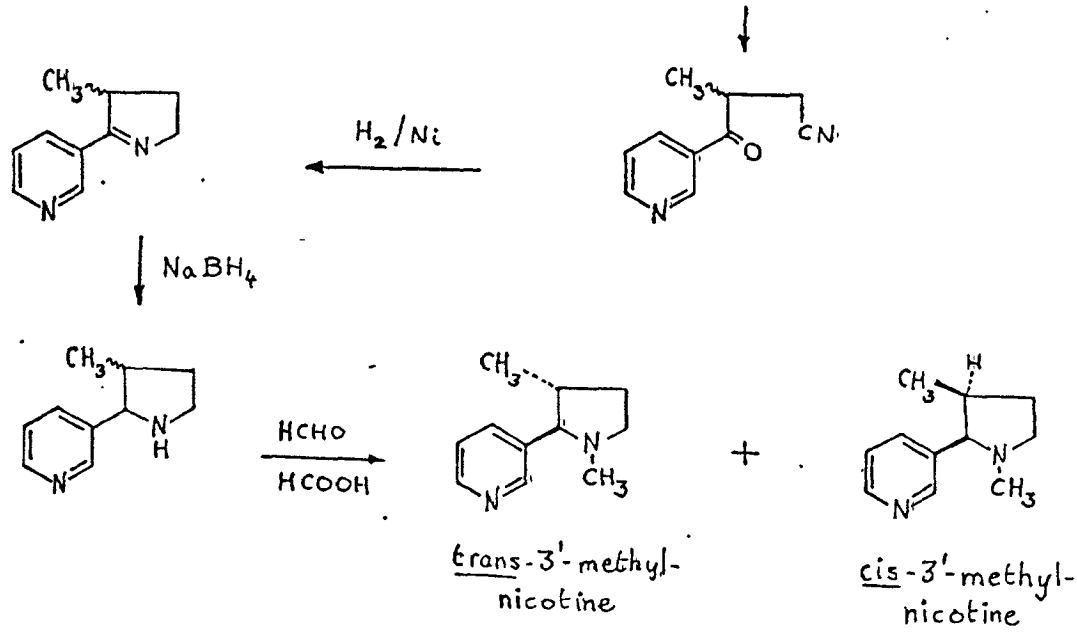
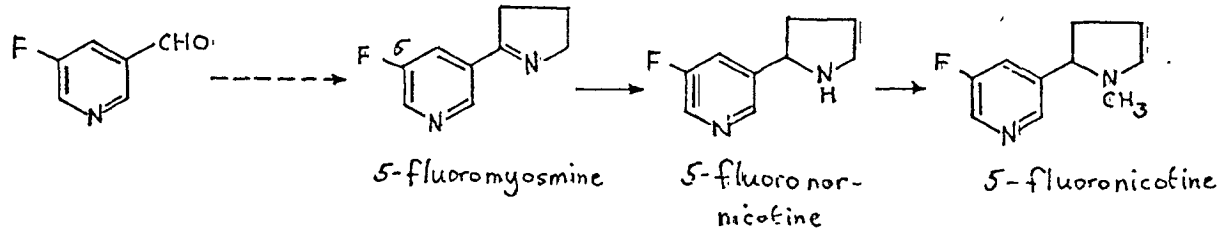


Other Nicotine Analogs

By the use of derivatives of pyridine-3-aldehyde in the above synthetic sequences it will be possible to prepare nicotine analogs with substitution in the pyridine ring. For example by the use of 5-fluoro-pyridine-3-aldehyde 5-fluoro derivatives of myosmine, normicotine, and nicotine will be prepared :



The fluorine group, being strongly electron attracting would be expected to reduce the basic strength of the pyridine nitrogen. This may have a profound effect on the binding properties of nicotine to the receptor site and its biological properties . We have prepared (RS)-5-fluoro-nicotine by another route (E. Leete, M. F. Manuel , and G B. Bodem, Phytochemistry, 10, 2687 (1971)) and this analog is currently being tested by Von Euler .

Source: https://www.industrydocuments.ucsf.edu/docs/jqvm0000

Item 9 (continued)

Biological Testing of the Nicotine Analogs

I have been in communication with Professor Holger Erdtman who is also interested in the problem of nicotine activity. He and his colleague Professor U. S. v. Euler at the Karolinska Institute, Stockholm, Sweden, are eager to examine the stereochemically pure methyl-nicotines which we propose to make . The synthesis of additional analogs will depend on the pharmacological results obtained by v. Euler . The compounds are tested in the following biological systems using l-nicotine as a standard.

1. Isolated rabbits jejunum
2. The guinea-pig ileum
3. The blood pressure of the cat
4. The isolated rectus abdominus muscle of the frog Rana temporaria

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The chemistry department at the University of Minnesota has well equipped laboratories for all types of chemical research. All modern instruments are available to aid in elucidation of structures, especially the stereochemistry of organic molecules. The equipment available to the principal investigator includes the following : MS-30 mass spectrometer with computor output, Varian XL-100 NMR spectrometer, Nuclear Chicago Mark II liquid scintillation counter, several IR, UV and ORD (Cary 60) spectrophotometers, several gas chromatograms, a Waters high pressure liquid chromatography system. The principal investigator has an active research group (currently 10 graduate students and one postdoctorate fellow) . Most of this group is housed in a new building which was opened in April 1971.

11. Additional facilities required:

None

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12. Biographical sketches of investigator(s) and other professional personnel (append):

Edward Leete (principal investigator), George B. Bodem (graduate student)
Philip Hoekstra (graduate student)

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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Item 12 (continued)

Biographical sketches of the professional personnel

Edward Leete, Principal investigator

REDACTED . Obtained a State Scholarship and attended the University of Leeds , obtaining a 1st class honours B.Sc. degree in Colour Chemistry and Dyeing in 1948 . Carried out graduate work in the department of colour chemistry with Professor William Bradley obtaining a Ph.D. degree in 1950 . Thesis title : Mechanism of the formation of indanthrone from 2-aminoanthraquinone. Awarded a travelling scholarship of the Goldsmiths Company and spent two years at the National Research Council of Canada, Ottawa, working with Dr. Leo Marion on alkaloids. In 1952 awarded an NRC postdoctoral fellowship and continued an additional two years with Leo Marion. In 1954 accepted a faculty position at the University of California, Los Angeles in the department of chemistry, instructor 1954-56 , assistant professor 1956-58 . In 1958 accepted a position at the University of Minnesota, assistant professor 1958-60. associate professor 1960-63, professor 1963- . REDACTED REDACTED . 1962-65 - member of the medicinal chemistry study section of the NIH. 1962-65 Alfred P. Sloan Foundation fellow. 1965 — Guggenheim fellow - held at the University of Oxford , England. 1965 — awarded D.Sc. degree from the University of Leeds. Notable invited lectureships : NSF lecturer at the University of Arizona, October 1967; Foster lecturer at the University of Buffalo 1969; Symposium lecturer at the 1st Philip Morris Science Symposium 1973 . Author of 131 scientific publications.

George B. Bodem, graduate student

REDACTED A resident of Minnesota where he went to Blake school. Obtained a B.S. degree from the University of Minnesota in 1962 . He has been a graduate student in my research group for several years and obtained an M.S. degree in 1972. Thesis title : Aberrant metabolism in higher plants : Formation of 5-fluoronicotine from 5-fluoronicotinic acid in Nicotiana tabacum. He is currently a Ph.D. candidate. He is an active and enthusiastic worker and has published 4 papers with the principal investigator. REDACTED

Philip M. Hoekstra, graduate student

REDACTED Obtained a B.A. degree from Dordt College, Sioux Center, Iowa in 1971 . He is currently a 2 nd year graduate student and he is a Ph.D. candidate having passed all the written preliminary examinations . He is currently studying the biomimetic synthesis of nicotine analogs . REDACTED

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Item 13 (continued)

Recent publications (reprints are attached at the end of this application)

E. Leete, M. R. Chedekel, and G. B. Bodem Synthesis of Myosmine and Nornicotine, Using an Acyl Carbanion Equivalent as an Intermediate J. Organic Chem., 37, 4465 (1972).

E. Leete, Biomimetic Synthesis of Nicotine. J. Chem. Soc., Chem. Commun., 1091 (1972).

E. Leete and A. R. Pinder, Biosynthesis of Dioscorine, Phytochemistry, 11, 3219 (1972).

E. Leete and M. R. Chedekel, The Aberrant Formation of (-)-N-Methyl-Anabasine from N-Methyl-Δ^1-piperideinium Chloride in Nicotiana tabacum and N. glauca, Phytochemistry, 11, 2751 (1972).

E. Leete and J. O. Olson, Biosynthesis and Metabolism of the Hemlock Alkaloids, J. Amer. Chem. Soc., 94, 5472 (1972).

E. Leete, Chapter 5 in "Biosynthesis" , A specialist Periodical Report of the Chemical Society, London, Edited by T. A. Geissman, 1972 , Biosynthesis of Alkaloids pp. 158-240.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)

	% time	Amount
Edward Leete (one month summer salary)	100	
George B. Boden (research assistant)	100	
Philip Hoekstra (research assistant)	100	REDACTED
Fringe Benefits on the above salaries (calculated at 16.1 %)		

Technical

REDACTED

Sub-Total for A

B Consumable supplies (by major categories)

Chemicals (organic compounds, solvents etc.)	3500
Glassware	1500

Sub Total for B 5000

C Other expenses (itemize)

Analytical Services (mass spectra NMR, CHN)	600
Page charges for journal articles	300
Travel to one domestic scientific meeting	300

Sub Total for C 1200

Running Total of A + B + C REDACTED

D Permanent equipment (itemize)

None

Sub Total for D 0

E Indirect costs (15% of A+B−C) E 3066

Total request $ REDACTED

15 Estimated future requirements

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2	REDACTED	5000	1200	0	3180	REDACTED
Year 3		0	1200	0	3300	

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Metabolism of Natural Products of Medicinal Interest	NIH-GM-13246-16	$ 47154	1.1.73-12.31 73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Renewal of the above NIH grant	NIH-GM-13246-17 (This will be the terminal year of a 5 year grant period)	$ 55510	1.1.74- 12.31.74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator
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Signature [signature] Date 7. 25.73
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University of Minnesota

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Responsible officer of institution
Typed Name Luther J. Pickrell
Title Assoc. Dean, Graduate School Res. Center
Signature [signature] Date
Telephone 612 (Area Code) 376 (Number) 7614 (Extension)

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